Award Number: W81XWH-14-1-0141

TITLE: Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease

PRINCIPAL INVESTIGATOR: Jonathan Chernoff, M.D., Ph.D.

CONTRACTING ORGANIZATION:

The Research Institute of Fox Chase Cancer Center
Philadelphia, PA 19111

REPORT DATE: June 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3 DATES COVERED
June 2017	Annual	15May2016-14May2017

4. TITLE AND SUBTITLE

Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-14-1-0141

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The major goal of this research project was to genetically and pharmacologically test the requirement of PAK signaling in Nf2 deficient schwannoma genesis. We planned to accomplish this goal by (1) using genetically intercrossed Pak1, Pak2 and a dominant negative Group A PAK transgene with our *PostnCre;Nf2*$^{flox/flox}$ mouse schwannoma model in order to generate functional (ABR testing) and histological readouts. These readouts allow us to utilize a genetic approach to determine if PAK signaling is essential to the development of *Nf2*-deficient schwannomas; (2) using histological and ABR readouts, we will treat *PostnCre;Nf2*$^{flox/flox}$ mice with three different pharmacologic PAK inhibitors to determine if targeted PAK inhibition in a preclinical model of schwannoma genesis rescues tumor development.

By the end of year two, we completed crosses of *Pak1*$^{-/-}$ and *Pak2*$^{flox/flox}$ mice with *PostnCre;Nf2*$^{flox/flox}$ mice and analyzed the effects of Pak loss on hearing and tumor growth. As we had speculated, loss of Pak1 function partly prevented hearing loss and tumor growth. Studies on Pak2 were also complete, and showed that loss of this kinase did not affect tumor growth. Treating these mice with Pak small molecule inhibitors was less successful, and we are currently attempting to determine the reasons why.

15. SUBJECT TERMS

NF2, Pak1, Pak2, PID, ABR, Pak inhibitors

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unlimited	Unclassified	UU	16	19b. TELEPHONE NUMBER *Include area code)*

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INTRODUCTION

Neurofibromatosis type 2 (NF2) is an autosomal dominant genetic disease characterized by benign schwannomas that grow on the cranial and spinal nerves. While technically benign, the tumors are nonetheless progressive and relentless, usually resulting in death before age fifty from inoperable intracranial masses. To date, surgery remains the only effective therapy for these lesions, though this therapy is frequently associated with major morbidities, including loss of hearing. In this award we proposed to evaluate Group A Paks (Pak1 and Pak2) as a therapeutic target in NF2 by intercrossing Group A PAK deficient mice with our NF2 mouse model (*PostnCre;Nf2$^{flox/flox}$*). Briefly, we hypothesized that the knockdown of Group A Paks in Nf2 deficient mice will rescue or reduce tumorigenesis. In **Specific Aim 1**, we proposed to characterize Pak's signaling influence on NF2 *in vivo* by assessing hearing thresholds, survival rate, tumor formation and kinome analysis. In **Specific Aim 2**, we proposed to test Pak inhibitors in our NF2 tumor model in order to evaluate whether the inhibition of Pak rescues merlin function to wild-type levels by analyzing readouts generated from tumor growth and auditory brainstem response (ABR).

KEYWORDS: NF2, Pak1, Pak2, PID, ABR, Pak inhibitors

ACCOMPLISHMENTS

(A) Major Goals of the Project

The major goal of this research project was to genetically and pharmacologically test the requirement of Group A PAK signaling in Nf2 deficient schwannoma genesis. We would accomplish this goal by (1) using our genetically intercrossed Pak1, Pak2 and a dominant negative Group A PAK transgene with our *PostnCre;Nf2$^{flox/flox}$* mouse schwannoma model in order to generate functional (ABR testing) and histological readouts. In turn these readouts will allow us to utilize a genetic approach to determine if indeed PAK signaling is essential to the development of *Nf2*-deficient schwannomas; (2) using histological and ABR readouts, we will treat *PostnCre;Nf2$^{flox/flox}$* mice with three different pharmacologic PAK inhibitors to determine if targeted PAK inhibition in a preclinical model of schwannoma genesis rescues tumor development.

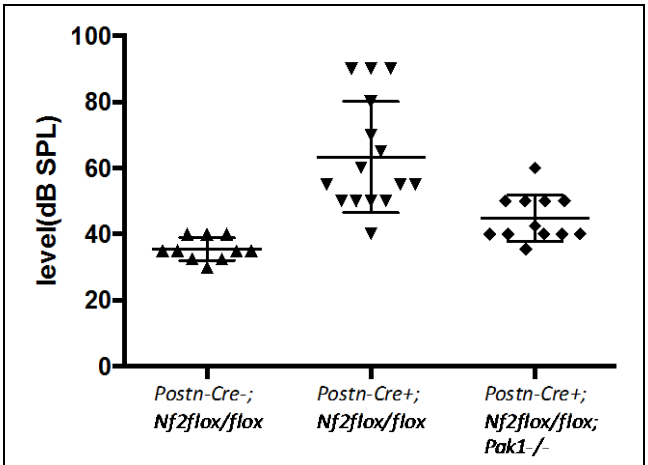

Figure 1. ABR study quantitating from 9-month- old Cre-negative control, Postn-Cre+; Nf2$^{flox/flox}$, Postn-Cre+; and Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/- mice, respectively. Each dot represents the average in left and right threshold of an individual ($P < 0.0001$).

(B) Accomplishments under these Goals

I n **Specific Aim 1A.1**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) by intercrossing these mice with our NF2 mouse model, *Postn-Cre; Nf2$^{flox/flox}$* to generate the following cohorts of mice; 15 *Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{+/+}$* mice (Control), 15 *Postn-Cre+; Nf2$^{flox/flox}$; Pak1$^{+/+}$* mice (NF2-KO), 15 *Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{-/-}$* mice (PAK1-KO), and 15 *Postn-Cre+; Nf2$^{flox/flox}$; Pak1$^{-/-}$* (NF2/PAK1-DKO). These cohorts of mice were utilized in order to assess hearing loss via auditory brainstem response (ABR) testing. Preliminary data included in the initial grant proposal indicated that our *Postn-Cre+; Nf2$^{flox/flox}$* mice showed by eight months of age, complete hearing loss, as compared to control mice, *Postn-Cre-; Nf2$^{flox/flox}$*. Functionally, the mean hearing threshold of nearly 60 dB in *Postn-Cre+; Nf2$^{flox/flox}$* mice at the age of 10 months is equivalent, as a human analog, of the inability to hear in a normal conversation, thus indicating severe disruption of an affected individual's life. In the analysis of 9 to 10 month old *Postn-Cre+; Nf2$^{flox/flox}$;Pak1$^{-/-}$* mice we observed that these mice showed improved hearing as compared 9 to 10 month old Nf2 deficient mice (*Postn-Cre+; Nf2$^{flox/flox}$* mice), as shown in **Figure 1**. Control

mice (*Postn-Cre-; Nf2$^{flox/flox}$; Pak1$^{+/+}$*) showed no hearing loss at 9 to 10 months of age. We have ended the ABR testing at 10 months of age for the *Pak*1 experimental cohorts and their *Nf2* deficient litter mates enrolled into the study as the *Nf2* deficient mice was having dramatic hearing loss, experiencing significant pain due to tumor burden and their survival rate was rapidly decreasing after 10 months of age. Therefore, after consulting with our colleagues, we decided to end this study for humane and ethnical reasons at 10months of age for all enrolled Pak1 deficient and their Nf2 deficient littermates enrolled into the study. From the data collected at 10 months of age, we can conclude that upon genetically knocking out Pak1 in Nf2 deficient mice improves hearing loss.

In the analysis of 4 to 6 months old *Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{-/-}$ we have observed that these mice showed no changes in hearing thresholds as compared to Postn-Cre+; Nf2$^{flox/flox}$* mice cohorts (data not shown). However, as discussed below, there are challenges in long-term evaluation of this strain due to the formation of new tumors.

In **Specific Aim 1A.2**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) in the NF2 mouse model by assessing tumor formation. Our preliminary data indicate that genetic disruption of Pak 1 or Pak 2 is not completely sufficient to inhibit tumor initiation (**Figures 2 and 3**). However, there is a strong but not statistically significant suggestion that genetic disruption of Pak1 is resulting in a reduction in dorsal root ganglia size as illustrated in **Figure 2**. Though we need to evaluate additional specimens, the histology of the dorsal root ganglia (DRG) of mice containing a disruption of the Pak1 allele appears more benign than the *Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$* and the *Postn-Cre+; Nf2$^{flox/flox}$*. However, more studies will be required to verify this observation. We will continue to analyze DRG volume in 10, 12 and 14 month old *Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/-, Postn-Cre-; Nf2$^{flox/flox}$;Pak1-/-, Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$, Postn-Cre-; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$ and Postn-Cre+; Nf2$^{flox/flox}$* in the no cost extension period.

In **Specific Aim 1A.3**, we proposed to characterize the role of individual Pak isoforms (Pak1 and Pak2) in our NF2 mouse model, *Postn-Cre; Nf2$^{flox/flox}$*, by assessing survival rate. Preliminary data included in the initial grant proposal indicated that our *Postn-Cre+; Nf2$^{flox/flox}$* mice showed by eight months of age, a decrease in survival as compared to control mice, *Postn-Cre-; Nf2$^{flox/flox}$*. By 20 months of age, roughly less than 40% of the Nf2/Pak1 deficient mouse cohort survival as compared to roughly 15% NF2 deficient cohort

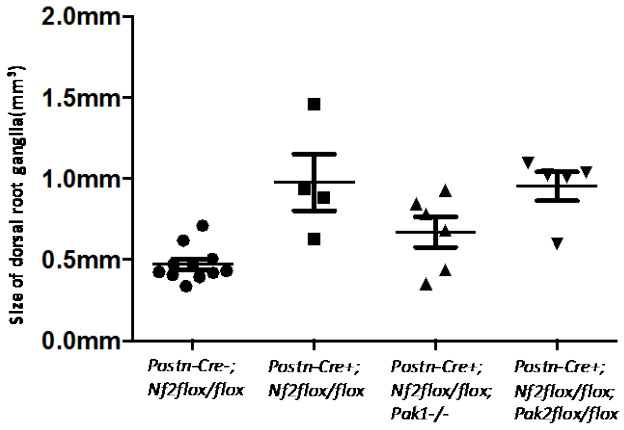

Figure 2. Mean size of dorsal root ganglia (DRG) from 10-month-old Cre-negative control, Postn-Cre+; Nf2$^{flox/flox}$, Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$ and Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/- mice, respectively. Each dot represents the size of an individual DRG. The line indicates the mean size of all DRG ($P < 0.1212$).

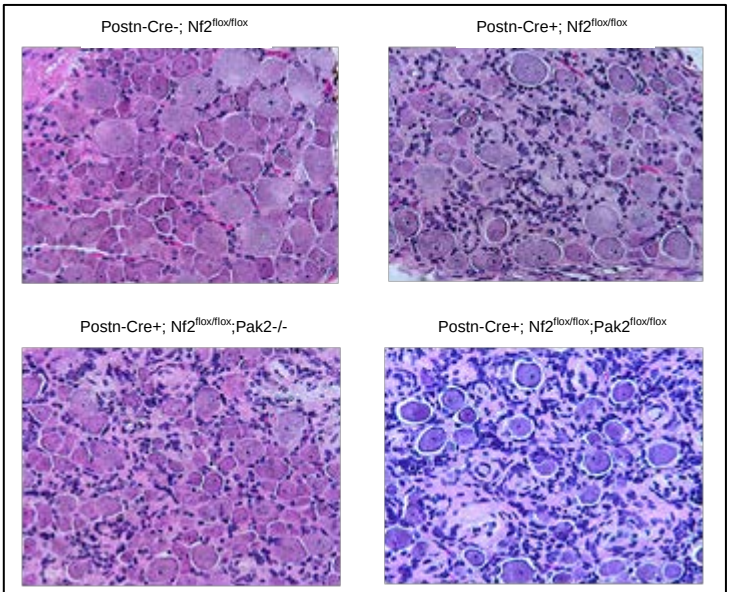

Figure 3. Hematoxylin and eosin (H&E) stain of the DRG from 10-month old Cre-negative control, Postn-Cre+; Nf2$^{flox/flox}$, Postn-Cre+; Nf2$^{flox/flox}$;Pak2$^{flox/flox}$ and Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/- mice, respectively. Postn-Cre+; Nf2$^{flox/flox}$;Pak1-/- mice slowly develop schwannomas od the DRG and spinal nerves compared to Postn-Cre+; Nf2$^{flox/flox}$.

Diffuse spinal nerve hyperplasia and psudo-onion bulb formation of the proliferating schwann cells observed in the nerve of Postn-Cre+; Nf2$^{flox/flox}$; Pak1-/ mice however frequency is lower. Original magnification x40.

survival (**Figure 4**). This data indicates that genetically knocking out Pak1 in Nf2 deficient mice rescues overall survival as compared to Nf2 deficient cohorts. No significant changes of survival rate was observed in the *Nf2$^{flox/flox}$;Pak1$^{+/+}$, Postn-Cre- (WT)* cohort of mice.

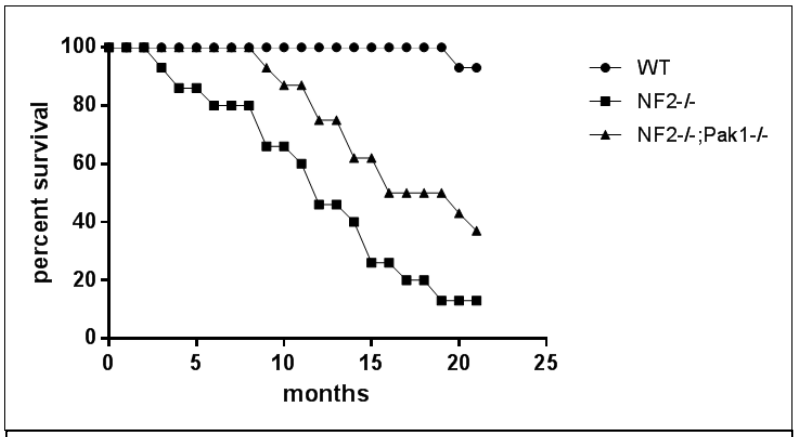

Figure 4. A Kaplan-meier plot of percent survival (Y axis) as a function of time (X axis) is show, Groups are Cre-negative control (n=15), Postn-Cre+;Nf2$^{flox/flox}$ (n=15) and Postn-Cre+;Nf2$^{flox/flox}$;Pak1-/- (n==16), respectively.

Currently, we have cohorts of *Nf2$^{flox/flox}$* and *Pak2*$^{flox/flox}$ enrolled in survival studies and during the no cost extension period we will continue to observe these cohorts. The enrolled cohorts are 15 *Postn-Cre-; Nf2$^{flox/flox}$; Pak2$^{flox/flox}$* mice (Control) and 15 *Postn-Cre+; Nf2$^{flox/flox}$; Pak2$^{flox/flox}$* (NF2/PAK2-DKO). Additionally, upon intercrossing the *Postn-Cre-; Nf2$^{flox/flox}$ with Pak2$^{flox/flox}$ mice in order to generate the Nf2$^{flox/flox}$; Pak2$^{flox/flox}$* mice (PAK2-KO),we observed by 2 months of age, these mice developed hind limb paralysis and had a body score of 1. Therefore, upon consultation with our animal facilities veterinarian and colleagues, we decided to end the survival studies for the genetically modified single knockout cohorts *Postn-Cre+; Nf2$^{+/+}$; Pak2$^{flox/flox}$* mice (Pak2-KO) and *Postn-Cre+; Nf2$^{Flox/Flox}$; Pak2$^{+/+}$* mice (Nf2-KO).

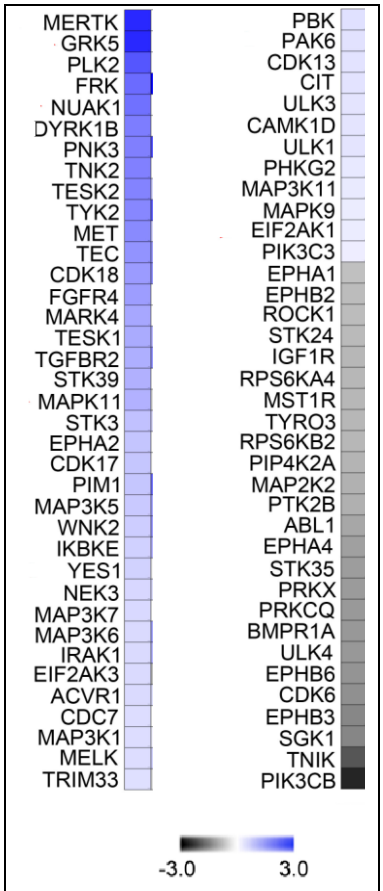

Figure 5. Preliminary MIBs analysis of tumors from Postn-Cre+; Nf2$^{flox/flox}$ *vs.* Postn-Cre+; Nf2$^{flox/flox}$;Pak1$^{-/-}$ mice. Ratios of kinase activity (Pak1$^{-/-}$/WT) are shown, with blue representing elevated activity, black representing diminished activity.

In **Specific Aim 1B,** we currently are intercrossing the planned cross of *Postn-Cre*; *Nf2$^{flox/flox}$* mice with ROSA26-LSL-PID mice and analyze tumor growth and hearing loss (Months 6-30). These experiments are ongoing.

In **Specific Aim 1C** we proposed to assess kinome activity in tumors from the following cohorts of mice: *Postn-Cre; Nf2$^{flox/flox}$* Pak1$^{-/-}$, and *Postn-Cre; Nf2$^{flox/flox}$* Pak2$^{flox/flox}$, and ROSA26-LSL-PID mouse models. Currently, we have preliminary data from the first of these crosses, *i.e.*, *Postn-Cre; Nf2$^{flox/flox}$* vs *Postn-Cre; Nf2$^{flox/flox}$*; Pak1$^{-/-}$ mice. Tumors were collected post ABR testing (performed in Aim1A.1) starting at 10 months of age, and at 12 months and at 14 months.

We pooled protein lysates derived from three schannomas from *Postn-Cre*; *Nf2$^{flox/flox}$*; *Pak1$^{+/+}$ and* three *Postn-Cre*; *Nf2$^{flox/flox}$*; *Pak1$^{-/-}$* mice, respectively, and subjected these lysates to MIBs analysis. The results of this preliminary screen are shown in Figure 5. We found elevated kinase activities for MERTK (a receptor tyosine kinase of the AXL family), GRK5 (a G Protein-Coupled Receptor Kinase), PLK2 (polo-like kinase), etc. (**Figure 5**). We are currently validating these findings using phospho-specific antibodies.

In **Specific Aim 2** we proposed to test small molecule Pak inhibitors on our *Postn-Cre; Nf2*$^{flox/flox}$ mouse model. During this reporting period, we completed these studies using FRAX-1036, a specific inhibitor of group A Paks. The compound was well tolerated in our murine Schwannoma model at the 30mg/kg/d dose and demonstrated robust inhibition of Pak phosphorylation *in vivo* (**Figure 6**).

Despite robust inhibition of Pak phosphorylation, tumor size was not significantly decreased in FRAX-1036 treated mice. ABR analysis revealed a modest decrease in hearing threshold after 12 weeks treatment versus vehicle control treated mice, but the decrease was not statistically significant (**Figure 7**).

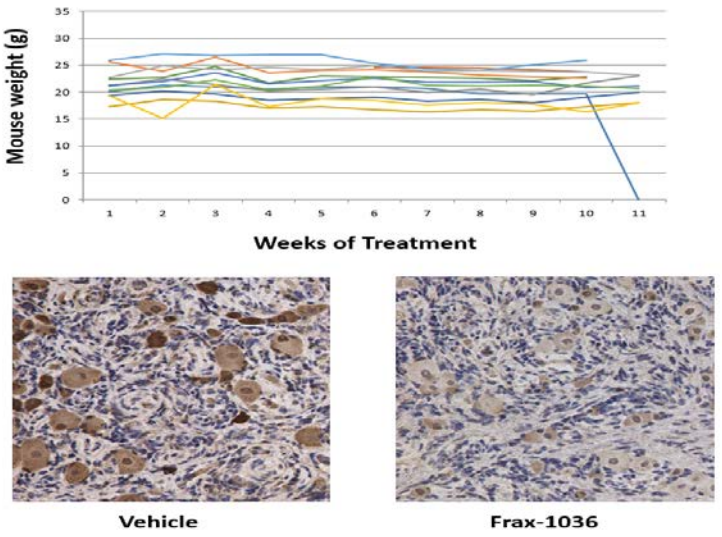

Figure 6. Mouse weights from FRX-1036 treated mice (top). IHC staining of DRGs using anti-phospho Pak (bottom).

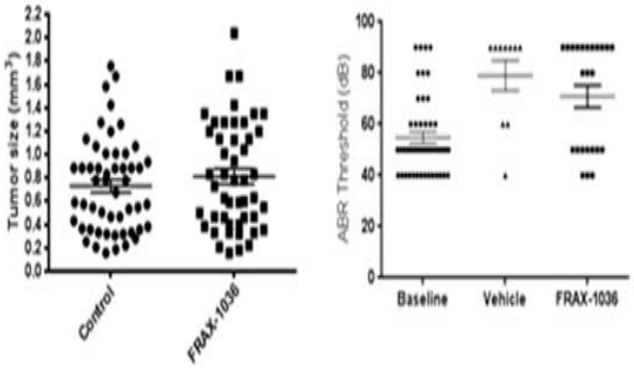

Figure 7. (a) DRG Size from FRAX-1036 and vehicle treated controls. Each dot represents an individual DRG. (b) Baseline and post-treatment ABR thresholds from FRZ-1036 and vehicle treated mice. Each dot represents an individual ear measurement.

(C) Training and Professional Development Opportunities

Nothing to Report

(D) Disseminated Results to Communities of Interest

Nothing to Report

(E) Plan for Next Reporting period

In order to accomplish the goals and objects stated above, during the no cost extension we will complete the genetic and ABR testing on Pak2 and PID cohorts, and complete kinome readouts and confirmatory data using phospho-specific antibodies.

IMPACT

Nothing to Report

CHANGES/PROBLEMS

Aim 1A.1: We have ended the ABR testing at 10 months of age for the *Pak1* experimental cohorts and their *Nf2* deficient litter mates enrolled into the study as the *Nf2* deficient mice was having dramatic hearing loss, experiencing significant pain due to tumor burden and their survival rate was rapidly decreasing after 10 months of age. Therefore, after consulting with our colleagues, we decided to end this study for humane and ethnical reasons at 10months of age for all enrolled Pak1 deficient and their Nf2 deficient littermates enrolled into the study. From the data collected at 10 months of age, we can conclude that upon genetically knocking out *Pak1* in Nf2 deficient mice improves hearing loss.

Aim 1A.3: Upon intercrossing the *Postn-Cre-; $Nf2^{flox/flox}$ with $Pak2^{flox/flox}$ mice in order to generate the $Nf2^{flox/flox}$; $Pak2^{flox/flox}$* mice (PAK2-KO), we observed by 2 months of age, these mice developed hind limb paralysis and had a body score of 1. Therefore, upon consultation with our animal facilities veterinarian and colleagues, we decided to end the survival studies for the genetically modified single knockout cohorts *Postn-Cre+; $Nf2^{+/+}$; $Pak2^{flox/flox}$*.

PRODUCTS

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	***J. Chernoff, M.D., Ph.D.***
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-4803-7836
Nearest person month worked:	*1*
Contribution to Project:	*Overall administration and guidance of research; Management and training of personnel*
Funding Support:	*N/A*
Name:	***H-Y Chow, Ph.D.***
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Kinome screening using MIBs; Examining tumor tissues by IHC and immunoblot*
Funding Support:	*N/A*
Name:	***D. Wade Clapp, M.D.***
Project Role:	Subcontract PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Oversee the laboratory infrastructure as well as the experimental studies performed in his laboratory*
Funding Support:	*N/A*
Name:	***Su-Jung Park, Ph.D.***
Project Role:	Associate Professor
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*In vivo studies; analysis of data sets collected from in vivo studies*
Funding Support:	*N/A*
Name:	***Li Jiang, BME***
Project Role:	Research Tech
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*ABR testing, mouse husbandry, PCR analysis and collect tissue samples post-morterm*
Funding Support:	*N/A*

SPECIAL REPORT REQUIREMENTS

Not applicable

APPENDICES

Not applicable

OTHER SUPPORT

Chernoff, Jonathan
Remaining salary support from institutional sources.

CURRENT

R01 CA142928 (PI: Chernoff) 1/1/2015 - 12/31/2019 20.0%
NIH 2.40 calendar
Targeting the Kinome in Neurofibromatosis Type 1
The major goals of this project are to determine: 1) What is the status of the kinome in NF1-/- Schwann cells and mast cells, and how does this change upon Mek, Akt/mTOR, or Pak inhibition?; 2) What is the cellular basis for Pak's function in NF1-related tumors? and 3) Are small molecule Pak inhibitors effective in preclinical models of NF1? Can whole kinome analysis predict pathways for drug resistance in treated mice?
Procuring Contracting/Grants Officer: Candace Cofie, 9609 Medical Center Dr., Bethesda, MD 20892, 240-276-6317

(PI: Chernoff) 2/1/2016 - 1/31/2018 5.0%
BCA 0.60 calendar
Dissecting a Breast Cancer Amplicon using CRISPR/Cas
This grant is in a one year extension.
The major goal of this project is to use a single pool of CRISPR-Cas constructs targeting all 198 genes in the 11q13.5-14.1 cluster between (and including) CCND1 and GAB2, and determine the relative contribution of each gene to the proliferation and survival of an IntClust-2 breast cancer cell line versus a non Intclust-2 breast cancer cell line.
Procuring Contracting/Grants Officer: Sharon Phillips, 48 Maple Ave., Greenwich, CT 06830, 203-861-0014

W81XWH-14-1-0141 (PI: Chernoff) 5/15/2014 - 5/14/2018 10.0%
DOD 1.20 calendar
Testing the Role of p21 Activated Kinases in Schwannoma Formation Using a Novel Genetically Engineered Murine Model that Closely Phenocopies Human NF2 Disease
The major goals of this project are: 1) Does Pak signaling influence NF2-related pathology in vivo?; and 2) Are small molecule inhibitors of Pak effective in preclinical models of NF2?
Procuring Contracting/Grants Officer: Sandra Rosario, US AMRAA, 820 Chandler St., Fort Detrick, MD 21702, sandra.rosario2.civ@mail.mil

P30 CA006927 (PI: Fisher) 8/12/2016 - 7/31/2021 25.0%
NIH 3.00 calendar
Comprehensive Cancer Center Program at Fox Chase
The major goal of this Cancer Center Support Grant is to provide partial salary support for professional personnel, including senior and program leadership, administration, planning and evaluation, and developmental funds, as well as support for 5 established peer-reviewed Research Programs, 12 Shared Research Resources and 2 Support Elements.
Procuring Contracting/Grants Officer: Candace Cofie, 9609 Medical Center Dr., Bethesda, MD 20892, 240-276-6317

R01 NS066927 (PI: Li, Vanderbilt Univ.) 3/1/2015 - 10.0%
2/29/2020 NIH 1.20 calendar
Pathophysiology of Conduction Block in HNPP
This project is a subcontract to Vanderbilt University.
Procuring Contracting/Grants Officer: Roddy Smith, Neurology Dept, 1161 21st Ave. South, Nashville, TN 37203, 615-936-8950

IRG-15-175-21 (PI: Chernoff) 1/1/2016 - 12/31/2018 NA
ACS
Institutional Research Grant
This is an Institutional Grant from the American Cancer Society that provides start-up funds for promising junior investigators to initiate innovative laboratory, clinical and translational research.
Procuring Contracting/Grants Officer: Virginia Krawiec, Extramural Grants Dept., 350 Williams St., Atlanta, GA 30303, 404-329-5734

R01 CA148805 (PI: Chernoff / Testa) 4/22/2016 - 3/31/2021 10.0%
NIH 1.20 calendar
Role of STE20 Protein Kinases in Malignant Mesothelioma
The major goals of this project are to: 1) Define the response of MM mice to Pak small molecule inhibitors and identify how tumors adapt to such inhibitors in vivo; and 2) Delineate the role of the Hippo pathway in Merlin-related signaling and MM pathology in vivo.
Procuring Contracting/Grants Officer: Candace Cofie, 9609 Medical Center Dr., Bethesda, MD 20892, 240-276-6317

T32 CA009035 (PI: Chernoff) 9/15/2016 - 8/31/2021 NIH 8.3%
1.00 calendar
Training Program in Cancer Research
The overall goal of this program is to prepare postdoctoral scientists for independent careers in basic and/or translational research focused on cancer.
Procuring Contracting/Grants Officer: Mutema Nyankale, 9609 Medical Center Dr., Bg. 9609, Rm 2W420, Rockville MD 20850, 240-2765987

COMPLETED
W81XWH-15-1-0192

OVERLAP
None

OTHER SUPPORT

CLAPP, D. WADE

CURRENT

K12 HD068371 (PI: Clapp) 03/01/13-11/30/17 5%
NIH/NICHD 0.60 Calendar Months

Indiana Pediatric Scientist Award (IPSA)

Goals and Aim: Leveraging an existing strong foundation of training, mentorship and research, the Indiana University School of Medicine (IUSM) Indiana Pediatric Scientist Award (IPSA) program will accelerate the success of junior faculty in bridging the gap between training and independence. The program will provide our junior faculty with a blueprint for success, structured mentorship and guidance and progressive goals in order to achieve independent funding within a five year period.

Role: Principle Investigator

Program Officer: Karen Winer, 6100 Executive Bvld., Room 4B11, MSC 7510, Bethesda MD, 20892

W81XWH-12-1-0155 (PI: Korf, UAB) 05/15/13-05/15/18 1%
DOD, University of Alabama, Birmingham 0.12 Calendar Months

NFRP Neurofirbromatosis Clinical Trials Consortium

Goals: Implement the clinical proposal; collect and transmit the data at Riley Hospital for Children at Indiana University Health. Participate in results preparation of the NF Consortium Infrastructure for presentation and publication.

Specific Aims:

Primary Aim: To estimate the objective response rate to Cabozantinib at 12 months in adults with NF1 plexiform neurofibromas by volumetric MRI imaging.

Secondary Aims:

1. To further assess the tolerability and toxicity of Cabozantinib in patients with NF1
2. To estimate the objective response rate of up to 2 non-target plexiform neurofibromas to Cabozantinib by MRI
3. To determine the quality of life (QOL) response to Cabozantinib in patients with NF1 plexiform neurofibromas
4. To access activity of Cabozantinib on mast cell activity by mast cell culture and FACS
5. To describe changes by flow cytometry in peripheral blood monocyte counts, circulating endothelial cells, and plasma angiogenic factors during treatment with Cabozantinib
6. To describe the baseline and change in 17 circulating cytokine factors related to proliferating cells
7. To characterize the pharmacokinetic profile of Cabozantibin in this population

Role: Site PI (Overall PI: Bruce Korf)

Program Officer: Naba Bora, 1077 Patchel St. Fort Detrick, Frederick, MD, 21702-9218

(PI: Clapp) 05/01/14-12/30/17 2.5%
Children's Tumor Foundation 0.30 Calendar Months

Experimental Therapeutics targeting the NF2 kinome: an integrated rational approach

Goals: The goal of this application is to bring a multidisciplinary group of investigators
together in a vertically integrated program that allows consistency of screening approaches, utilizes both established and newly developed cellular and murine models of NF2-associated tumors, allows validation of the mechanisms of action of drugs and investigation of the biologic result of target inhibition in both VS and meningiomas as well as exploration of the cellular compensatory events influencing drug response with the ultimate goal of developing clinical studies that have the highest chance of success for patients.

Specific Aims:

Aim 1: Utilize NF2/(Nf2)-deficient schwannoma and meningioma cells to screen:

a) Established FDA-approved drugs and targeted therapeutics in the clinical pipeline that have rational linkage to Nf2-dependent pathways in collaboration with industry partners

b) A library of late-stage and FDA-approved drugs in collaboration with the National Center for Advancement of Translational Science (NCATS) to allow an unbiased drug screen

Aim 2: Utilize genetically engineered (VS) and xenograft (meningioma) models to test drugs that meet in vitro go/no go criteria:

a) Vestibular schwannomas: PeriostinCre; Nf2flox/flox mice will be treated with drugs successful in the in vitro screen against schwannomas. The preclinical endpoints will be restoration of baseline hearing in 50% of mice and 50% mean reduction in paraspinal tumor volume by MRI and at autopsy in 75% of mice compared to mice treated with a vehicle control.

b) Meningiomas: Ben-Men-1 cells (benign NF2-deficient meningioma cells) will be implanted intracranially and mice will be treated with drugs successful in the in vitro screen against meningiomas. The preclinical endpoint will be 50% mean reduction in tumor volume by MRI and luciferase activity in 75% of mice compared to mice treated with a vehicle control.

Aim 3: Identify at least 1 drug to move forward for a clinical trial.

a) Drs. Blakeley and Welling will be involved in all stages of go/no-go decision making and will design the appropriate clinical trial for the agent(s) with the best portfolio based on:

a) Acceptable safety profile

b) Efficacy in vitro and in vivo against cell culture models of schwannoma and meningioma - Preference will be for joint activity against VS and meningioma and for drugs with delineated mechanisms in NF2 tumors

c) Drugs that are in active clinical development with an industry partner

Aim 4: Delineate cell and molecular validation of the best performing experimental compounds

a) Identify cellular mechanisms of action of drugs in tumors in vitro and in vivo

b) Utilize immunohistochemistry and western blot to validate inhibition of the biochemical target in tumorigenic cells of the in vivo models

c) Conduct kinome analyses on drugs that pass the established go/no go criteria for at least one in vivo model to evaluate patterns of adaptation to therapy and the differences between meningioma and VS therapeutic responses

d) Confirm synthetic lethality via genetic confirmation with shRNA

Role: Multi-PI (David W. Clapp, Jaishri Blakeley, Scott Plotkin and James Guesella)

Program Officer: Annette Bakker, 95 Pine Street, 16th Floor, New York, NY, 10005

W81XWH-14-1-0141 (PI: Chernoff, FCCC) 05/14/14-05/13/18 1%

DOD/NFRP 0.12 Calendar Months

Testing the Pharmacologic and genetic role of P21 activated kinase inhibition on Schwannoma Formation using a Novel Genetically Engineered Murine Mouse Model that Closely Phenocopies Human NF2 Disease

Goals: The objective of this project is to evaluate Pak as a therapeutic target in NF2.

Specific Aims:

Aim 1: Does Pak signaling influence NF2-related pathology *in vivo*? If group A Paks are important for growth and motility signaling in cells lacking NF2, then loss of Pak function should slow or prevent pathologies associated with loss of the *Nf2* gene in mice. We have already constructed $Pak1^{-/-}$ and $Pak2^{flox/flox}$ mice, as well a targeted transgenic knock-in mouse that conditionally expresses the PID, a peptide inhibitor of all group A Paks, in any tissue expressing Cre recombinase. We will cross these transgenic mice, as well as our existing *Pak1* knock-out mice, with Periostin (*Postn*)-*Cre*; $Nf2^{flox/flox}$ mice to answer the following questions

a) Does loss of the *Pak1* or *Pak2* gene alone ameliorate loss of the *Nf2* gene in schwannoma formation? What signaling pathways mediate these effects?

b) Does suppression of group A Pak kinase function by a genetically encoded peptide inhibitor ameliorate loss of the *Nf2* gene *in vivo*? What signaling pathways mediate these effects?
c) What is the basal state of the kinome in NF2 schwannomas and how is the kinome in Schwann cells or tumors reprogrammed upon loss of Pak function?

Aim 2: Are small molecule inhibitors of Pak effective in preclinical models of NF2? Selective small molecule Pak inhibitors have recently entered clinical trials. In NF2 cell culture and in xenograft settings, such small molecule inhibitors have proven effective in preventing tumor growth and in inhibiting Merlin signaling. We will test three of the most advanced Pak inhibitors to determine if they are beneficial in improving auditory function in *Postn-Cre; Nf2* $^{flox/flox}$ mice and in reducing tumor growth:

a) Do inhibitors of group A Paks restore normal hearing and/or promote tumor regression in *Postn-Cre*; *Nf2*$^{flox/flox}$ mice?
b) Do pan-Pak (group A plus group B) inhibitors restore normal hearing and/or promote tumor regression in *Postn-Cre*; *Nf2*$^{flox/flox}$ mice?

Role: Co- Investigator (PI: Jonathan Chernoff)
Program Officer: Scott Linton, U.S. Army Medical Research and Material Command, Fort Detrick, Maryland, 21702-5012

U54 CA196519 (PI: Clapp/Shannon) 09/01/15-08/31/20 20%
NIH/NCI 2.40 Calendar Months

Developmental HyperActive Ras Tumor SPORE

Goals: The overall goal of this SPORE is to implement effective targeted molecular therapies for neoplasms and cancers characterized by *NF1* mutations by conducting integrated, mechanistically based translational research. This highly-qualified, collaborative group will address the following overarching objectives:

1. To evaluate novel therapeutics in validated preclinical models and in the treatment of patients with NF1.
2. To Identify risk factors of individuals with NF1 to acquire spontaneous and treatment-associated second malignancies
3. To decrease tumor associated morbidity and mortality of patients with NF1

Role: Multi-PI (David W. Clapp and Kevin Shannon)
Program Officer: Igor Kuzmin, 9609 Medical Center Drive, Room 3W112, MSC 9726, Bethesda, MD, 20892

(PI: Ferrer) 09/01/15-08/31/18 10%
DOD 1.20 Calendar Months

Discovery of drug combinations for the treatment of Neurofibromatosis malignancies

Goals: To identify compounds that can inhibit signaling pathways regulated by Ras in NF1
Specific Aims:
Aim 1: High Throughput (HTS) screening of a library of chemotherapeutic agents to identify drugs that induce cells death of plexiform NF1-/- Schwann Cells.
Aim 2: Dose Response Matrix screening to identify synergistic drug combinations in plexiform NF1-/- Schwann Cells.
Aim 3: Determination of therapeutic effects of synergistic combinations in inhibiting tumor growth in mouse models of pNF1.
Role: Co-Investigator (PI: Marc Ferrer)
Program Officer: Akua Roach, U.S. Army Medical Research and Material Command, Fort Detrick, Maryland, 21702-5012

T32 DK007519 (PI: Broxmeyer) 07/01/85-06/30/18 NA
NIH/NIDDK NA

Regulation of Hematopoietic Cell Production

Goals: The goal of this application is to continue training the next generation of scientists in the clinically-relevant medical area of the regulation of hematopoietic cell production.
Role: Mentor

Program Officer: Terry R. Bishop, The National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 20892-2560

(PI: Morrison) 08/16/16-06/30/17 1%
Leibniz Institute on Aging- Fritz Lipmann Institute, Germany 0.12 Calendar Months
Testing the Pharmokenetic effect of Neuregulin 1 (NRG1) antibody has on the Genesis of Plexiform Neurofibromas and Vestibular Schwannomas.
Goals: To Test the effect KTN3379 antibody has on Schwann Cells and the genesis of plexiform neurofibromas
Specific Aims:
Aim 1: The first study will be to test the efficacy of NRG1 in a small cohort of plexiform neurofibroma and vestibular schwannoma (N=6 per mouse model) as a pilot study, prior to moving into a larger efficacy study. Post 90 day treatment, mice will be euthanized and tumor size and number will be evaluated. Histological and Immunohistochemistry analysis will be conducted as well in looking at phospho-Erk levels.
Aim 2: Upon evaluating the data collected in Aim 1 and if a reduction of tumor size, tumor number and pERK is shown we will repeat these studies in a larger efficacy study (n=12 mice/group) and treat for 90 days.
Role: Co-Investigator (PI: Helen Morrison)
Program Officer: Helen Morrison, Beutenbergstrabe 11, D-07745 Jena, Germany

NF150083 (PI: Shekhar) 05/01/16-04/31/19 10%
DOD 1.20 Calendar Months
Molecular Mechanisms and Therapeutics Development for Social and Communication Learning Deficits in NF1
Goals: The goal of this application is to further elucidate the molecular mechanisms underlying the behavioral and communication disruptions exhibited by *Nf1*+/- mice and test novel therapeutic approaches to treating these debilitating symptoms seen in subsets of NF1 patients.
Specific Aim I: To determine the neural networks and molecular mechanisms involved in the social and communication deficits seen with NF1 mutation.
Aim 1A: Map the brain networks and molecular mechanisms involved in the social learning and ultrasonic vocalization communications in *Wt* versus $Nf1^{+/-}$ mice utilizing pERK immunocytochemistry (ICC) and NARG gene expression panels.
Aim 1B: Demonstrate the functional organization of the putative brain networks for behavioral deficits seen in $Nf1^{+/-}$ mice by optogenetically modulating their function through stimulating or inhibiting identified neurons in the relevant pathways.
Specific Aim II: To test if blocking *Ras-MAPK over* activity by targeting two different downstream molecular targets with novel pharmacological inhibitors will rescue the deficits in $Nf1^{+/-}$ mice.
Role: Investigator (PI: Anantha Shekhar)
Program Officer: Danielle Reckley, 820 Chandler Street, Bldg. 843, Fort Detrick, MD 21702-5014

(PI: Clapp) 09/01/16-08/30/17 2%
Neurofibromatosis Therapeutics Acceleration Program (NTAP) 0.24 Calendar Months
Testing the Pharmacologic role of Molecular Target inhibition on Plexiform Neurofibromas in a Genetically Engineered Mouse Model that closely Phenocopies Human Neurofibromatosis Type 1
Goals: The goal of this application is to continue our efforts in investigating the pathogenesis and treatment of NF1 patients afflicted with plexiform neurofibromas.
Specific Aims:
Aim 1: Test the efficacy of AAV serotypes against existing plexiform neurofibroma development.
Aim 2: Test the efficacy of Pazopanib (VEGFR inhibitor) as a single therapeutic agent as well as in combination with M1043 (monoclonal antibody against endoglin) against existing plexiform neurofibroma development.
Aim 3: Test the efficacy of LY2157299 (TGF-β receptor Type 1 inhibitor) as a single therapeutic agent against existing plexiform neurofibroma development.
Role: Principal Investigator
Program Officer: Jaishri Blakeley, 600 N. Wolfe St., Baltimore, M.D, 21287

(PI: Clapp) 04/01/17-11/30/17
Takeda Development Centre Europe Inc. 3%
0.36 Calendar Months

Evaluation of the NAE inhibitor MLN3651, as a single agent, for treatment of vestibular schwannomas in preclinical models of Neurofibromatosis Type 2

Goals: The purpose of the research is to identify novel therapies for the treatment of vestibular schwannomas. Specifically, we will test the drug, MLN3651, a 2nd generation oral derivative of MLN4924 and a potent and selective Nedd8 Activating Enzyme (NAE) inhibitor targeting to restore $CLR4^{DCAF1}$ activity in Merlin mutant tumors. MLN3651 will be tested as a single agent in our NF2 mouse model and its therapeutic efficacy targeting vestibular schwannoma growth will be evaluated.

Program Officer: Mahindra Makhija, 61 Aldwych, London, WC2B 4AE, UK

COMPLETED

Children's Tumor Foundation – "Drug Discovery Initiative Neurofibromatosis Preclinical Consortium Center"
R42 HL099150

OVERLAP

None